

Application No. 09/766,113
Reply to Office Action dated March 17, 2003

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of the claims in the application:

Claim 24 is newly added.

Claims 1-10 are under examination.

Claims 11-23 have been withdrawn.

Listing of Claims:

1. (Currently Amended) A plant promoter comprising at least one tissue-preferred plant promoter element, said element identified by:
 - a) providing a first mixture of oligonucleotides each comprising a 5' flanking sequence, a central random sequence, and a 3' flanking sequence;
 - b) contacting said first mixture with a second mixture comprising nuclear proteins from a preferred plant tissue under binding conditions promoting complex formation between said oligonucleotides and said nuclear proteins, and allowing formation of said oligonucleotide-protein complexes;
 - c) separating said formed complexes from each other electrophoretically;
 - d) isolating said separated complexes in ranges of electrophoretic mobility and separating said oligonucleotides from said isolated complexes;
 - e) amplifying said separated oligonucleotides ~~of said isolated complexes~~ by polymerase chain reaction utilizing primers to said flanking sequences;

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- f) providing said amplified oligonucleotides from step e) as the first mixture for a repetition of step a);
- g) performing at least a second cycle of steps b-e with said provided oligonucleotides of step f);
- h) assessing for a particular range of electrophoretic mobility and quantity of complex formation in progressive cycles of step g);
- i) isolating oligonucleotides of a particular range of electrophoretic mobility wherein, over said progressive cycles, said range has increased complex formation ~~in step h)~~ relative to that in the original cycle;
- j) operably linking individual oligonucleotides of step i) to a promoter that drives expression in a plant cell, said promoter operably linked to a coding sequence in an expression cassette;
- k) allowing expression of said expression cassette and assessing tissue-preferred expression of said coding sequence; and
- l) choosing an determining sequence of an oligonucleotide having tissue-preferred expression in step k) and determining sequence of said oligonucleotide, which is a tissue-preferred plant promoter element.

2. (Currently Amended) The promoter of claim 1, wherein said preferred plant tissue is root tissue and said tissue-preferred promoter element is a root-preferred promoter element.

3. (Original) The plant promoter of claim 1 comprising at least one synthetic root-preferred plant promoter element that enhances expression of a coding sequence operably linked to said promoter.

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4. (Original) The plant promoter of claim 1 comprising at least one synthetic root-preferred plant promoter element that suppresses expression of a coding sequence operably linked to said promoter.

5. (Currently amended) A plant promoter comprising at least one root-preferred plant promoter element which functions in modulation of transcription of an operably-linked polynucleotide, comprising a nucleotide sequence selected from the group consisting of:

- a) a nucleotide sequence of ~~SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, or SEQ ID NO. 8;~~
- b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a) SEQ ID NO: 2, wherein stringent conditions comprise hybridization in 50% formamide, 1 M NaCl, 1% SDS at about 37°C, and a wash in 0.1X SSC at about 60 to about 65°C; and
- c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a) SEQ ID NO: 2, wherein said contiguous nucleotides maintain function of the nucleotide sequence of a) SEQ ID NO: 2.

6. (Currently amended) A chimeric gene DNA of interest comprising the promoter of claim 5 operably linked to a nucleotide coding sequence of interest.

7. (Currently amended) An expression cassette comprising the chimeric gene DNA of interest of claim 6.

8. (Original) A transformation vector comprising the expression cassette of claim 7.

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9. (Original) A transformed plant having stably incorporated into its genome the transformation vector of claim 8.

10. (Currently amended) A plant promoter comprising at least one multimeric root-preferred promoter element which functions in modulation of transcription of an operably-linked polynucleotide, comprising at least two root-preferred promoter elements further comprising a nucleotide sequence selected from the group consisting of:

- a) a nucleotide sequence of ~~SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, or SEQ ID NO. 8;~~
- b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a) SEQ ID NO: 2, wherein stringent conditions comprise hybridization in 50% formamide, 1 M NaCl, 1% SDS at about 37°C, and a wash in 0.1X SSC at about 60 to about 65°C; and
- c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a) SEQ ID NO: 2, wherein said contiguous nucleotides maintain function of the nucleotide sequence of a) SEQ ID NO: 2.

11. (Withdrawn) A plant promoter comprising at least one root-preferred plant promoter element that enhances expression of a coding sequence operably linked to said promoter, wherein said element comprises a nucleotide sequence selected from the group consisting of:

- a) a nucleotide sequence of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, or SEQ ID NO. 8;

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- b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a); and
- c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a), wherein said contiguous nucleotides maintain function of the nucleotide sequence of a).

12. (Withdrawn) A plant promoter comprising at least one root-preferred plant promoter element that suppresses expression of a coding sequence operably linked to said promoter, wherein said element comprises a nucleotide sequence selected from the group consisting of:

- a) a nucleotide sequence of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, or SEQ ID NO. 8;
- b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a); and
- c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a), wherein said contiguous nucleotides maintain function of the nucleotide sequence of a).

13. (Withdrawn) A transformed plant, or its parts, having stably incorporated into its genome a DNA construct comprising a plant promoter operably linked to a coding sequence, said plant promoter comprising at least one synthetic root-preferred plant promoter element.

14. (Withdrawn) The plant, or its parts, of Claim 13 wherein said element comprises a nucleotide sequence selected from the group consisting of:

- a) a nucleotide sequence of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, or SEQ ID NO. 8;

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- b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a); and
 - c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a), wherein said contiguous nucleotides maintain function of the nucleotide sequence of a).
15. (Withdrawn) The plant, or its parts, of claim 13, wherein said plant is a dicot.
16. (Withdrawn) The plant, or its parts, of claim 13, wherein said plant is a monocot.
17. (Withdrawn) The plant, or its parts, of claim 16, wherein said monocot is maize.
18. (Withdrawn) The plant of claim 13, wherein said plant expresses a DNA coding sequence operably linked to said promoter.
19. (Withdrawn) A transformed plant cell, said plant cell having stably incorporated into its genome a DNA construct comprising a plant promoter operably linked to a coding sequence, said plant promoter comprising at least one synthetic root-preferred plant promoter element.
20. (Withdrawn) A method for root-preferred expression of a nucleotide coding sequence in a plant, said method comprising transforming a plant cell with a transformation vector comprising an expression cassette, said expression cassette comprising a plant promoter operably linked to said nucleotide coding sequence, said plant promoter comprising at least one synthetic root-preferred plant promoter element.

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21. (Withdrawn) The method of claim 20 wherein said element comprises a nucleotide sequence selected from the group consisting of:

- a) a nucleotide sequence of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, or SEQ ID NO. 8;
- b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a); and
- c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a), wherein said contiguous nucleotides maintain function of the nucleotide sequence of a).

22. (Withdrawn) A method for identifying and isolating tissue-preferred promoter elements, said method comprising the steps of:

- a) providing a first mixture of oligonucleotides each comprising a 5' flanking sequence, a central random sequence, and a 3' flanking sequence;
- b) contacting said first mixture with a second mixture comprising nuclear proteins from a preferred plant tissue under binding conditions promoting complex formation between said oligonucleotides and said proteins;
- c) separating said formed complexes electrophoretically;
- d) isolating said separated complexes in ranges of electrophoretic mobility;
- e) amplifying oligonucleotides of said isolated complexes by polymerase chain reaction utilizing primers to said flanking sequences;
- f) providing said amplified oligonucleotides from step e) as the first mixture for a repetition of step a);

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- g) performing at least a second cycle of steps b-e with said provided oligonucleotides of step f);
- h) assessing for a particular range of electrophoretic mobility and quantity of complex formation in progressive cycles of step g);
- i) isolating by cloning, individual oligonucleotides of a particular range of electrophoretic mobility wherein said range has increased complex formation in step h);
- j) simultaneous with step i) or as an individual step, operably linking isolated individual oligonucleotides of step i) to a promoter that drives expression in a plant cell, said promoter operably linked to a coding sequence in an expression cassette;
- k) assessing tissue-preferred expression of said coding sequence; and
- l) determining sequence of an oligonucleotide having tissue-preferred expression in step k).

23. (Withdrawn) The method of claim 22 further comprising assessing binding affinity of an individually cloned oligonucleotide of said isolated oligonucleotides of step i) for nuclear proteins from said preferred plant tissue of step b).

24. (New) The promoter of claim 1, wherein said preferred plant tissue is *Zea mays* root tissue and said tissue-preferred promoter element is a root-preferred promoter element.